IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	McCart et al.
Appl. No.	:	09/991,721
Filed	:	November 13, 2001
For	:	COMBINED GROWTH FACTOR-DELETED AND THYMIDINE KINASE-DELETED VACCINIA VIRUS VECTOR
Examiner	:	Sullivan, Daniel M.
Group Art Unit	:	1636

DECLARATION UNDER 37 CFR 1.132 OF DAVID L. BARTLETT, M.D. and BERNARD MOSS, M.D., Ph.D.

We, David L. Bartlett, M.D. and Bernard Moss, M.D., Ph.D., do hereby declare:

1. We are named inventors of the above-identified application. True and correct copies of our Curriculum Vitae are attached as Exhibit 1 (Dr. Bartlett) and Exhibit 2 (Dr. Moss).

2. As described in our patent application, and published as the post-filing date art of McCart et al. (Dec 2001) Cancer Res 61: 8751, of record, to enhance the tumor specificity of vaccinia virus, we developed a combined thymidine kinase-deleted (TK-) and vaccinia growth factor-deleted (VGF-) vaccinia virus and investigated its properties *in vitro* and *in vivo*. The gene for enhanced green fluorescent protein (EGFP) was inserted into the *TK* locus of a VGF- vaccinia virus by homologous recombination creating a double-deleted mutant vaccinia virus (vvDD). A WR strain vaccinia virus that had the *lacZ* gene inserted into its VGF sites was used as the parent virus. A vaccinia shuttle plasmid was created that allowed for homologous recombination of EGFP into the *TK* locus of the parent virus, creating vvDD.

3. Infection of resting and dividing NIH3T3 cells with vvDD yielded reduced viral production compared with wild-type (WT), TK-, or VGF- viruses from resting cultures but equivalent virus production from dividing cultures. After nude mice were injected i.p. with WT, TK-, VGF-, or vvDD vaccinia virus, tissues and tumor were harvested for viral titer determination. As illustrated by Fig. 5 of our patent application, no virus was recovered from the brains of mice injected with vvDD compared with the other viruses; however, equivalent amounts were recovered from tumor.

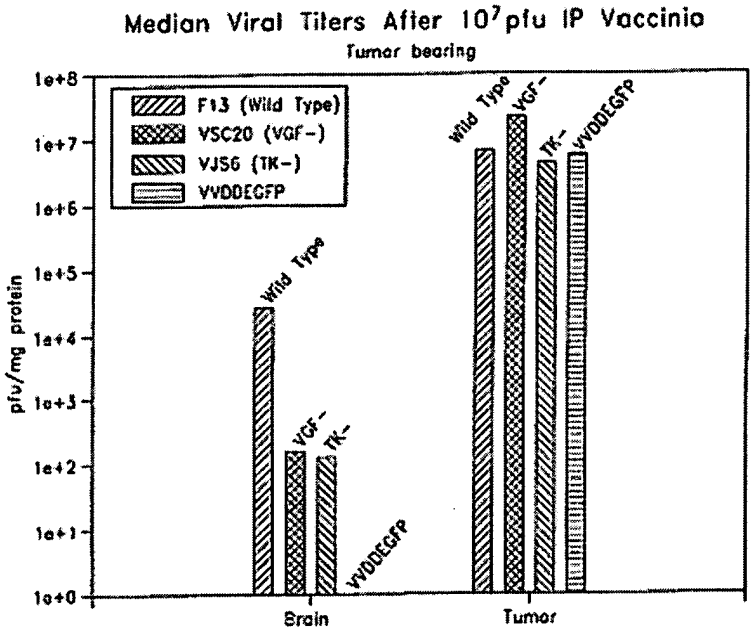

FIG. 5

4. There was no apparent toxicity from vvDD because nude mice receiving vvDD lived long-term, whereas mice receiving WT, VGF-, or TK- virus had median survivals of only days. As illustrated by Fig. 6 of our patent application, nude mice bearing s.c. murine colon adenocarcinoma (MC38) had significant tumor regression after treatment with systemic (i.p.) vvDD compared with control.

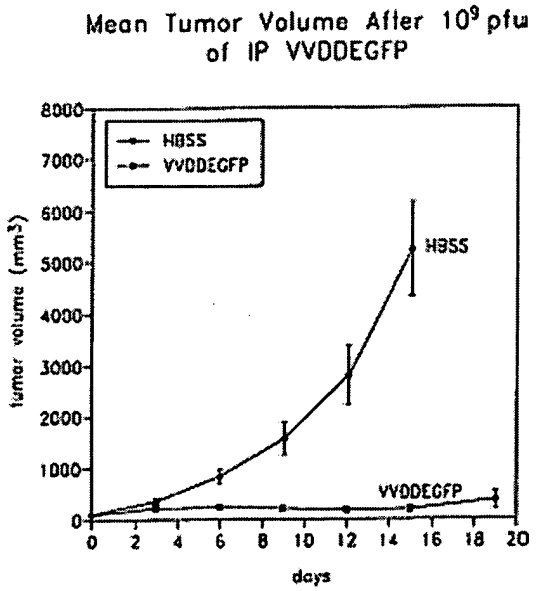

FIG. 6

5. Remarkably, this antitumor effect is attributable to the replication of virus alone because no therapeutic genes had been included.

6. Our data demonstrate that a TK- and VGF- mutant vaccinia virus is significantly attenuated in resting cells *in vitro* and demonstrates tumor-specific replication *in vivo*. It is a remarkable vector for use in tumor-directed gene therapy, given its enhanced safety profile, tumor selectivity, and the oncolytic effects after systemic delivery.

7. International Prosecution: The International Preliminary Examination Report (IPER) purported that the concept underlying the inventions is not novel over WO 92/15672 (**D1**). According to the IPER, **D1** discloses recombinant vaccinia viruses vP938 (= NYVAC.2) and vP977 (both in example 17 on pages 124-129), as well as vP996 (example 69 on page 281), which have a thymidine kinase (TK) and vaccinia growth factor (VGF) negative phenotype.

8. The IPER has also alleged that the claims lack inventive step. The argument is that the invention could have been made in an obvious way by combining **D1** with any of EP 0 443 335 (**D2**) or WO 94/16716 (**D3**). According to the IPER, **D2** discloses plasmids for generation of recombinant poxviruses via insertion of a transgene in the thymidine kinase locus and provides incentive to use attenuated vaccinia viruses for expressing transgenes, including such with inactivated VGF gene. Also according to the IPER, **D3** provides vaccinia vectors for immunotherapy and discloses attenuated thymidine kinase negative NYVAC vectors expressing the tumor suppressor p53, as well as NYVAC vectors expressing cytokines.

9. Nevertheless, the vaccinia virus expression vector provided in **D1** comprises many different mutations (more than 40 ORFs deletions) and is too attenuated to replicate well in human cancer cells. By combining **D1** with any of **D2** or **D3**, the skilled artisan would not reach a method and a vaccinia virus expression vector according to the invention. Indeed, one would not infer from the cited references that the combination of the two mutations (TK and VGF) were sufficient for obtaining the effects sought, i.e., robust replication in human cancer cells and no viral replication in non-dividing cells. Therefore, the methods and the vaccinia virus expression vectors according to the invention are novel and inventive.

10. U.S. Prosecution: The U.S. Patent and Trademark Office (USPTO) rejected the claims under 35 USC 103(a) as being unpatentable over Mastrangelo et al. (1995) WO 95/31105 in view of Dorner et al. USP 6,103,244 and further in view of Buller et al. (1988) J Virol 62: 866; additional claims further in view of Zhang et al. (1996) Biochem Biophys Res Commun 227: 707; and further claims further in view of Paoletti USP 5,942,235 (as further evidenced by P04637).

11. Unsuggested Modification: The so-called prior art lacks any suggestion that the references should be combined in a manner required to meet the claims. One line of literature describes a VGF- virus. Another line of literature describes a TK- virus. While each method of engineering was designed to attenuate the vaccinia virus, nothing in the prior art suggested the desirability of combining the TK and VGF deletions. This is because, on the one hand, no additive effect might have been achieved. On the other hand, the combination may have restored over attenuation, as in NYVAC.2, which has a thymidine kinase (TK) and vaccinia growth factor (VGF) negative phenotype and more than 40 ORFs deletions. Ours is the first report of a mutant vaccinia virus with multiple selective mutations to enhance tumor specificity.

12. New Principle of Operation: The invention utilizes a new principle of operation. As explained in McCart et al. (Dec 2001), p. 8751, 2nd col., 2nd and 3rd full paragraphs, previously, deletion of either the *TK* gene or *VGF* genes was shown to significantly decrease pathogenicity compared with WT virus. A TK- virus requires TTP for DNA synthesis from the nucleotide pool present in dividing cells. This leads to preferential viral replication in dividing cells and is the presumed explanation for the observed tumor specificity. VGF is a secreted protein produced early in viral infection and acts as a mitogen to prime resting surrounding cells for vaccinia infection. Deletion of this growth factor causes decreased viral replication in resting cells and a 1000-fold increase in the LD_{50} of intracranial vaccinia. The combined effect of TK and VGF deletions on tumor specificity turned out to be synergistic. In the absence of TK, viral replication will require TTP from dividing cells. The normal stimulation of resting surrounding cells to divide will not occur in the absence of VGF; hence, replication will occur only in actively dividing cells.

13. Unexpected Results: Notwithstanding the statement in McCart et al. (Dec 2001), p. 8751, 2nd col., 3rd full paragraph "The combined effect of TK and VGF deletions on tumor specificity should be synergistic", the results achieved by the invention are new, unexpected, superior, disproportionate, unsuggested, unusual , critical, and surprising. This is because the comparative data show synergy, and synergism evidences nonobviousness.

14. As for our statement, "The combined effect of TK and VGF deletions on tumor specificity should be synergistic", it does not negative patentability. By our statement that "The

combined effect ... should be synergistic", the operative word is "should", and was written in hindsight. At the time of the invention, the combined effect could not be predicted based on the two deletions separately.

15. Not even we, as experts, could know what to expect based on the known properties of vaccinia virus or the role of the TK and VGF genes in viral infection. It is common sense that we had knowledge of our own work, Buller et al. (1988) J Virol 62: 866 (deletion of the *VGF* gene), of record, and Buller et al. (1985) Nature 317: 813 (deletion of the *TK* gene), attached. Nevertheless, as detailed above, one would not infer from the NYVAC.2 literature that the combination of the two mutations (TK and VGF) were sufficient for obtaining the effects sought, i.e., robust replication in human cancer cells and no viral replication in non-dividing cells. Also, as elaborated above, the combination may have restored over attenuation, as in NYVAC.2. There was nothing in the literature (not even our own work) to suggest a mutant vaccinia virus with multiple selective mutations to enhance tumor specificity. Hindsight reasoning permitted us to combine the various existing features of vaccinia viruses and the principles of TK and VGF in viral infection in a new way to explain the new result. We acknowledged, however, that a complete explanation for this tumor selectivity has yet to be elucidated. McCart et al. (Dec 2001), p. 8754, 2nd col., beginning of 1st full paragraph. We recognized, too, the hypothetical nature of our preliminary explanation in admitting "The hypothesis for the high level of tumor selectivity shown by the current vector is a combination of the two". McCart et al. (Dec 2001), p. 8754, 2nd col., ending of 1st full paragraph. Ultimately, as evidenced by the timing of McCart et al., which was published as post-filing date art, we drew the conclusion, as opposed to making a prediction, that "The combined effect ... should be synergistic" as retrospection because the combined effect seemed logical in retrospect but the prior art did not teach an expectation of the combined effect.

16. In conclusion, the statement "The combined effect of TK and VGF deletions on tumor specificity should be synergistic" was a conclusion and not a prediction, because, in sum, any additive effect of the two deletions could not be predicted in advance, thus, here, synergism, which is undisputed, evidences nonobviousness.

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I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Respectfully submitted,

Dated: 5/1/06

By: _____
David L. Bartlett, M.D.

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Respectfully submitted,

Dated: 4/27/2006 By: Bernard Moss

Bernard Moss, M.D., Ph.D.

AMEND

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